Influence of Ovarian Follicle Sizes and Estrous Signs on Pregnancy Following Progesterone-Based Fixed Time Artificial Insemination in Water Buffaloes

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Abstract: The objectives of the present study were to elucidate the importance of follicle sizes and estrous signs during Controlled Internal Drug Release-Synch-human Chorionic Gonadotropin (CIDR-Synch-hCG) protocol for Fixed Time Artificial Insemination (FTAI) and to evaluate their association with pregnancy in water buffaloes. Data from riverine buffaloes (n = 207) under the CIDR-Synch-hCG protocol were analyzed. Buffaloes were administered with Gonadotropin-Releasing Hormone (GnRH) with insertion of CIDR on Day 0. Prostaglandin ($PGF^2\alpha$) was given on Day 7 with the removal of CIDR. hCG was given on Day 9, and AI was performed on Day 10. Follicle measurements by ultrasonography were done on Days 0, 7, and 10, and follicle sizes on those days were categorized into I, II, and III. Estrus signs were taken on the day of AI. The pregnancy diagnosis was done on Day 30-35 post-AI. The average size of follicles in Category III is significantly higher than those of Categories I and II, regardless of the Days of the protocol. Pregnancy is significantly ($P<0.001$) associated with Pre-Ovulatory Follicle (POF) size and uterine tonicity on the Day of AI but not with follicle sizes on Days 0 and 7, nor with mucus discharge discharge ($P>0.05$). The overall pregnancy rate is 44.44% while performing AI with POF size ≥12.0 mm increased the probability of pregnancy rate to 56.25%. In conclusion, the present study demonstrated a follicle size-based CIDR-Synch-hCG protocol providing new fertility indicators to improve FTAI efficiency in buffaloes with huge application in other livestock species.

Keywords: Fixed time AI, follicle sizes, estrous signs, pregnancy, water buffaloes.

INTRODUCTION

Buffaloes are important animals in Asia, playing major roles in livestock production as a source of milk and meat, which has caught the interest of buffalo raisers worldwide to improve reproduction. The application of various reproductive biotechnologies represents the major intervention to improve reproduction and production in this species [1]. One major technology that is widely used in dairy cattle [2] and in buffaloes [3] is Fixed Time Artificial Insemination (FTAI) to address post-partum infertility and long calving interval, which are the main causes of poor reproductive efficiency in these species.

In 2014, the intensified reproductive management program in buffaloes was developed, dubbed as the Triple E Strategy, with the following components: Enhancing AI Efficiency, Early Pregnancy Diagnosis, and Effective Rebreeding Program to address reproductive concerns in buffaloes at the National Genepool of the Philippine Carabao Center [4]. Central to these strategies is understanding the reproductive physiology and ovarian dynamics, which resulted in enhanced AI efficiency and improved other reproductive biotechnologies. Initial local works on FTAI in buffaloes involved the optimization of the original Ovsynch protocol [5], which was consequently modified to find more effective protocols in water buffaloes. The supplementation of exogenous progesterone at the initiation of the FTAI program was found important in repeat or hard breeder buffaloes, resulting in the development of the CIDR-Synch protocol [6]. A further attempt to improve pregnancy was focused on the choice of the ovulatory hormone, from GnRH to human Chorionic Gonadotropin (hCG), for its direct action on the ovarian follicle for final maturation and ovulation. This led to the development of the CIDR-Synch-hCG protocol used in the present study.

Underpinning the reproductive physiological milieu and a deeper understanding of mechanisms and regulation of ovarian functions are essential to improve efficiencies of assisted reproductive tools in water buffaloes. Recently, the importance of ovarian structures at various time points of the FTAI program has been given attention as possible factors influencing pregnancy. Several studies have revealed that follicular

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size at estrus influences the luteal profiles and subsequent pregnancy rates in beef [3,7] and dairy cows [8]. The pregnancy rate was also greater in cows that had a larger follicle on the day of FTAI, which was associated with a greater display of estrus, ovulation rate, and pregnancy following AI [9]. An optimal size of 11-13 mm of ovulatory follicles resulted in positive benefits on the pregnancy rate in lactating beef cows [10]. The studies mentioned above in cattle clearly indicate that a particular size of the follicle during FTAI programs influences ovulation and pregnancy rates; however, such information is scanty and limiting in buffaloes [11,12]. The dual role of ovarian follicles in reproduction: production of competent oocytes for fertilization and steroidogenic functions during estrus, ovulation, and pregnancy must be further explored to achieve the desired efficiencies of timed AI programs. The present study, therefore, aimed to characterize the follicle sizes and signs of estrus and determine their association with pregnancy following CIDR-Synch-hCG FTAI protocol and to establish ovarian conditions at the time of AI as possible indicators of female fertility in water buffaloes.

MATERIALS AND METHODS

All works and procedures involving the use of animals for scientific research were followed in accordance with the requirements for the protection and welfare of animals of Philippine Animal Welfare Act of 1998 and was approved by the Ethics Committee for experimentation of the Philippine Carabao Center, Department of Agriculture.

Animal Selection and Induction of Ovulation for FTAI

This prospective study was carried out involving multiparous Murrah-based riverine dairy buffaloes (*n* =207) subjected to Controlled Internal Drug Release-Synch-human Chorionic Gonadotropin (CIDR-Synch-hCG) protocol from 2017-2021 at Philippine Carabao Center, National Genepool, Nueva Ecija, Philippines. Buffaloes were selected based on the following conditions: with a body condition score greater than 3.0 [13], not pregnant following ultrasound examination (HS-1600, Honda Electronics Co., Ltd. Japan), and with one or two ovaries with sizes greater than 1 cm. In addition, the presence of corpus luteum (CL) in the ovary was noted during animal selection to determine ovarian activity and animal cyclicity. Buffaloes were then subjected to CIDR-Synch-hCG protocol for FTAI (Figure **1**). In brief, buffaloes received 2 ml intramuscular (IM) injection of gonadotropin-releasing hormone (GnRH, Cystorelin, 50 ug/ml) simultaneously with intravaginal insertion of CIDR (Eazi-Breed, 1.38-g progesterone) on Day 0. CIDR was removed, and 5 ml of IM injection of prostaglandin ($PGF_2\alpha$, Lutalyse, 5 mg/ml) was done on Day 7. Two (2) ml of hCG (Chorulon, 1,000 I.U/ml) was given on Day 9 as an ovulatory hormone. AI was conducted twice using semen from a bull with known fertility at 14-16 and 22-24 hrs after hCG injection.

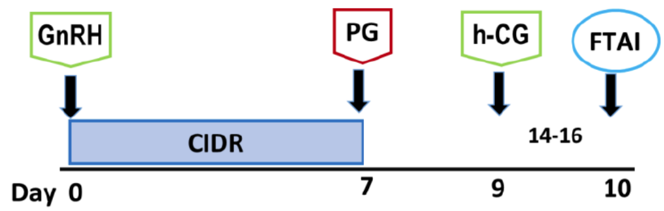

Figure 1: Schematic diagram of CIDR-Synch-hCG protocol in water buffaloes. Day 0: Injection of 2 ml GnRH and insertion of CIDR device. Day 7: Removal of CIDR and injection of 5 ml $PGF^2\alpha$. Day 9: Injection of 2 ml hCG. Day 10: Conduct of FTAI 14-16 and 22-24 hrs after hCG injection.

Follicle Size Measurement, Estrous Signs, and Experimental Design

The examination of ovaries and measurement of sizes of follicles present in both ovaries were performed using transrectal ultrasonography (HS-1600, Honda Electronics Co, Lt. Japan) on Days 0 and 7 of the protocol. Further, follicular development was monitored, and the size of Pre-Ovulatory Follicle (POF) was taken on the Day of AI at Day 10. Animals were likewise observed for estrous signs (uterine tone and mucus discharge) on the day of AI. Pregnancy diagnosis was performed by ultrasonography within Days 30-35 post-AI, and animals are considered pregnant based on the presence of amniotic fluid and conceptus in the uterus. Confirmation of pregnancy was done by rectal palpation on Day 60 post-AI. The pregnancy rate was defined as the number of buffaloes diagnosed as pregnant on days 30-35 divided by the number of buffaloes inseminated.

Dominant follicle (DF) sizes on Day 0 were categorized into Category 1: <8.0 mm and Category III: ≥10.0 mm, following the previous study with modification [14]. Follicles with <8.0 mm are considered as small and medium DF, while those with sizes ≥8.0 mm are considered as large DF under the present context. DF on Day 7 was classified the same as that of Day 0. Meanwhile, the sizes of POF taken on the Day of AI at day 10 were categorized into Category

I: <10.0 mm, Category II: 10.0 to 11.9 mm, and Category III: ≥12.0 mm, following earlier work with modification [11]. In addition, the signs of estrus manifested by the animals on the Day of AI were classified. For uterine tonicity, classifications were as follows: Tone 1 (considerably soft), Tone 2 (considerably hard), and Tone 3 (considerably very hard). Meanwhile, mucus discharges were classified as MD 1 (light and watery consistency), MD 2 (sticky consistency and glassy color), and MD 3 (very sticky consistency and glassy color).

Statistical Analysis

All statistical analysis was performed using JMP 13.2 statistical software (SAS Institute Inc., Cary, NC, USA) for Windows. Numerical average data on follicle sizes were presented as mean ± SE. The average follicle sizes among the three categories of follicles on Days 0, 7, and 10 were compared using the general linear model/ANOVA. Chi-square analysis was used to determine the association of follicle sizes with pregnancy outcomes following the CIDR-Synch-hCG protocol. Chi-square test analysis was used because the response variable (pregnant/not pregnant) is not a continuous variable but rather categorical. Thus, the analysis was used to determine if there were any significant differences in frequencies in pregnancy among the three follicle size categories (Category I – III). Logistic Regression Analysis was used to determine which among the factors, namely Follicle sizes on Days 0, 7, and 10 of the protocol, uterine tonicity, and mucus discharge on the day of AI, can influence pregnancy outcome. Data analysis on continuous variables with multiple fixed effects and covariates is often analyzed by multiple linear regression to determine which among the fixed effects significantly influence the response variable and to determine the strength of the linear relationship between the response variable and explanatory variable (fixed effects), if any. As the response variable in this study is not continuous but rather a categorical variable (pregnant/not pregnant), Logistic Regression analysis is more suitable. The Logistic Regression Analysis platform of JMP 13.2 software includes odds-ratio prediction that can determine the chances of pregnancy. Probabilities of less than 5% level were considered statistically significant ($P< 0.05$).

RESULTS

Characterization of Ovarian Follicles during CIDR-Synch-hCG Protocol in Dairy Buffaloes

Ovarian ultrasonography performed in 207 riverine buffaloes revealed the presence of follicles at various sizes in all animals examined on Days 0, 7, and 10 of the CIDR-Synch-hCG protocol. Follicles on Days 0 and 7 revealed similar average follicle sizes of around 9.0 mm, which is mainly considered a Dominant Follicle (DF). Likewise, follicle sizes for both Days ranged from 6.0 to 13.0 mm (Table **1**). With further development of DF to POF, an increase in the average size of follicles to 11.3 mm, ranging from 8.09 to 18.8 mm, was observed on Day 10 of the protocol. Follicle growth and development during the 10-day FTAI program defines follicular developmental stage and diameter.

Follicle Sizes on Day 0, Pregnancy Rate and their Association following CIDR-Synch-hCG Protocol in Dairy Buffaloes

Percent distribution of the treated animals to follicle size categories revealed a wide variability of follicle sizes at the initiation of the protocol (Table **2**). The average follicle sizes among categories differed significantly ($P<0.05$). Following insemination, follicle Category II yielded the highest pregnancy. However, the follicle sizes on Day 0 are not associated with pregnancy rate ($P=0.0814$).

Follicle Sizes on Day 7, Pregnancy Rate, and their Association following CIDR-Synch-hCG Protocol in Dairy Buffaloes

Injection of GnRH on Day 0 is expected to result in the emergence of a new follicular wave and the formation of new DF on Day 7, and treated buffaloes

Table 1: Descriptive Characterization of Ovarian Follicles following CIDR-Synch-hCG Protocol for FTAI in Water Buffaloes (N=207)

Days of FTAI program	Day 0	Day 7	Day 10
Follicle Size (mm)	9.00±1.52	9.14±1.24	11.26±1.75
Minimum	6.00	6.20	8.09
Maximum	13.0	12.9	18.8

Data on follicle sizes were presented as mean ± SE.

Table 2: Association of Follicular Sizes on Day 0 with Pregnancy following CIDR-Synch-hCG Protocol for FTAI in Water Buffaloes (n=207)

Measures	Follicular Size Categories			
	Category I (<8mm)	Category II (8-9.9mm)	Category III (≥ 10mm)	Total
Animal distribution, n (%)	74 (35.7%)	75 (36.2%)	58 (28.0%)	207
Ave. Follicle diameter, mm[1]	7.42±0.07[c]	9.13±0.07[b]	10.99±0.08[a]	
Pregnancy/Category, n (%)[2]	28/74 (37.8%)	41/75 (54.7%)	23/58 (39.7%)	92 (44.4%)

[1]Numerical average data on follicle sizes were presented as mean ± SE. Different letters (a–c) in the same row differ significantly (P < 0.05).
[2]Pregnancy is not significantly correlated with Follicle size on Day 0: ($\chi2$=5.02, Prob>ChiSq =0.0814, r=0.03).

Table 3: Association of Follicular Size on Day 7 with Pregnancy following CIDR-Synch-hCG Protocol for FTAI in Water Buffaloes (n=207)

Measures	Follicular Size Categories			
	Category I (<8mm)	Category II (8-9.9mm)	Category III (≥ 10mm)	Total n
Animal distribution, n (%)	65 (31%)	110 (53%)	32 (15%)	207
Ave. Follicle diameter, mm[1]	7.44±0.07[c]	9.06±0.05[b]	10.93±0.10[a]	
Pregnancy rate/Category, n (%)[2]	20/65 (30.8%)	62/110 (56.4%)	10/32 (31.2%)	92 (44.4%)

[1]Numerical average data on follicle sizes were presented as mean ± SE. Different letters (a–c) in the same row differ significantly (P < 0.05).
[2]Pregnancy is positively correlated with follicle size on Day 7: ($\chi2$=13.5084, Prob>ChiSq=0.0012, r=0.06).

were similarly distributed among the three follicle categories, with the majority observed under Category II (Table **3**). The average follicle sizes differed significantly (P<0.05) among the Categories. Similarly, Category II yielded the highest pregnancy rates. There is a significant association between the presence of DF follicle on Day 7 and pregnancy rate (P=0.0012).

Follicle Size on the Day of AI, Pregnancy Rate, and their Association following CIDR-Synch-hCG Protocol in Dairy Buffaloes

Ovarian examination conducted on Day 10 demonstrated that the majority of the animals were in Categories II and III with POF sizes of ≥10.0 mm (Table **4**). The average follicle sizes under three Categories differ significantly (P<0.05). The pregnancy rate in Category 2 is higher than Category 1, while Category III has the highest pregnancy rate. POF size on the Day AI is significantly associated with pregnancy (P=0.0007). Taken together, regardless of follicle size categories, the overall pregnancy rate of 44% was achieved following the CIDR-Synch-hCG protocol, and performing AI with a POF size of 13.3 mm resulted in the highest pregnancy (56.2%). Pregnancy is improved when buffaloes have a larger POF on the day of FTAI.

Estrous Signs and their Association with Pregnancy following CIDR-Synch-hCG Protocol in Dairy Buffaloes

Buffaloes observed for estrous signs on the Day of AI revealed the following: in terms of uterine tonicity, most of the buffaloes were found with Tone 3 and Tone 2, but none of the animals was with Tone 1 (Table **5**). Subsequently, animals with Tone 3 achieved a higher

Table 4: Association of Follicular Sizes on Day 10 with Pregnancy following CIDR-Synch-hCG Protocol for FTAI in Water Buffaloes (n=207)

Measures	POF Categories (mm)			
	Category I (<10mm)	Category II (10-11.9mm)	Category III (≥ 12mm)	Total n
Animal distribution, n (%)	34 (16.0%)	93 (45.0%)	80 (39.0%)	207
Ave. POF diameter[1]	9.28±0.20[c]	11.18±0.09[b]	13.33±0.10[a]	
Pregnancy rate/Category[2]	6/34 (17.6%)	41/93 (44.1%)	45/80 (56.2%)	
Overall Pregnancy rate	6 (2.9%)	41 (19.8%)	45 (21.7%)	92 (44.4%)

[1]Numerical average data on follicle sizes were presented as mean ± SE. Different letters (a–c) in the same row differ significantly (P < 0.05).
[2]Pregnancy is highly correlated with follicle size on the Day of AI: ($\chi2$=14.41, Prob>ChiSq =0.0007, r=0.254).

Table 5: Association of Estrous Signs and Pregnancy following CIDR-Synch-hCG Protocol for FTAI in Water Buffaloes (n=207)

Uterine Tone Category	Tone 1	Tone 2	Tone 3
Animal distribution, n (%)	0.0	96/207 (46.4%)	111/207 (54.0%)
Pregnancy/Category, n (%)[1]	0.0	21/96 (21.9%)	71/111 (64.0%)
Mucus Discharge Category	**MD 1**	**MD 2**	**MD 3**
Animal distribution, n (%)	66/207 (31.8%)	81/207 (39.1%)	60/207 (28.9%)
Pregnancy/Category, n (%)[2]	31/66 (47.0%)	37/81 (45.7%)	24/60 (40.0%)

[1]Pregnancy is highly associated with Uterine Tone: ($\chi 2$=63.93, Prob>ChiSq=0.0001, r=0.42).
[2]Pregnancy is not significantly correlated with Mucus Discharge: ($\chi 2$=0.07005, Prob>ChiSq=0.7045, r=0.054).

pregnancy rate compared with those with Tone 2. The uterine tone is highly associated with pregnancy (P=0.0001). Meanwhile, mucus discharge (MD) is variable and widely distributed among the 3 MD categories and is not significantly associated with pregnancy (P=0.7045). Uterine tonicity on the Day of AI is a significant estrous sign related to fertility and pregnancy in buffaloes.

Logistic Regression Analysis (Model 1), which was used to predict the effect of the factors on pregnancy outcome, namely follicle size on Days 0, 7, and 10, uterine tone, and mucus discharge, revealed that the use of follicle sizes on Days 0 and 7 as well as mucus discharge are not significantly associated with pregnancy outcome as shown in (Table **6**). A second Logistic regression analysis was done with only uterine tone and follicle size at Day 10 included (Model 2), suggesting a better model with an overall Prob>ChiSq = 0.0013 versus Prob>ChiSq = 0.0288 for Model 1.

Furthermore, Odds-Ratio (OR) prediction revealed chances of pregnancy with the follicle size on the Day of AI at Day 10 (OR = 1.2558, P<0.021) and uterine tonicity (OR= 5.02, P <0.0001). Follicle sizes on Days 0 and 7 and mucus discharge on the Day of AI (OR= 0.58, P <0.16) would not likely determine the pregnancy outcome.

In practical application, the decision as to whether to inseminate will be facilitated by the size of the follicle on the Day of AI at Day 10. The probability of pregnancy increases with follicle size on the Day of AI. The optimum follicle size to achieve a 0.55 – 0.75 probability of pregnancy should be between 12 mm and 16 mm in size and a uterine tone of 3.

DISCUSSION

The effect of Pre-Ovulatory Follicle size on fertility has been a subject of interest in timed AI in livestock over the past few years, and several studies have been produced in beef cattle [8,15,16] and buffaloes [11,12]. The inclusion of follicle sizes at earlier developmental stages before the Pre-Ovulatory Follicle stage in the present investigation was made to understand the importance of a particular size of the follicle during CIDR-Synch-hCG FTAI protocol in buffaloes.

The presence of large follicles on Day 0 is the major basis for a GnRH-based ovulation synchronization, which resulted in an effective response of Dominant Follicle to GnRH to induce ovulation and the

Table 6: Logistic Regression Analysis on Different Factors in Predicting Pregnancy in Dairy Buffaloes

Model 1 TERM	PARAMETER		Chi-Square	Prob>ChiSq
	Estimate	SE		
Intercept	-3.4996	1.4104	6.16	0.0131*
Follicle size, DAY 0	0.0410	0.1247	0.11	0.7422
Follicle size, DAY 7	-0.0547	0.1666	0.11	0.7425
Follicle size, DAY 10 (day of AI)	0.2413	0.1045	5.33	0.021*
Uterine Tone	1.7051	0.3331	26.21	<.0001**
Mucus Discharge	-0.5394	0.3841	1.97	0.1602

Follicle size on Day 10 and Uterine tone (Y = -7.009 + (0.2278*Day of AI fol. Size) +(1.6146*Uterine tone).

emergence of new follicular wave in cattle [2,5] and water buffaloes [17]. The induction of ovulation of large follicles on Day 0 in Mediterranean buffaloes with the Ovsynch protocol yielded a conception rate of around 50%, which conforms with the high pregnancy rate of DF under Category II in the present study [14]. Both works corroborate with evidence that the presence of a large follicle at the beginning of the GnRH-based protocol is a determining factor for a successful synchronization of ovulation and high conception rates. In the present study, the size of DF on Day 0, however, is not positively correlated with pregnancy since these follicles may not be the ones that will ovulate for insemination at the end of the protocol, but rather those which will ovulate and trigger the emergence of new follicle wave at the first GnRH injection. The effect of DF at Day 0 on the overall pregnancy can be described as indirect and important. Neglia *et al*. [17] stated that ovulation of DF at the start of the protocol subsequently optimizes physiological responses of the CL to PGF2a on Day 7 and the response of the POF to GnRH2 on Day 9, increasing the likelihood of pregnancy to Timed AI. In contrast, the low pregnancy outcome observed in Category I could be due to the poor response of smaller follicles ≤8.0 mm to GnRH ovulation induction.

A high pregnancy rate was similarly achieved from DF on Day 7 in Category II, suggesting the formation of new DF following induction of ovulation with the current protocol on Day 0. It is worth emphasizing that the DF formed on Day 7 are the ovulatory follicles that will eventually be subjected to timed insemination. In addition, the association of DF size on Day 7 with pregnancy conforms with earlier studies, implying a direct relationship between ovulation of DF after GnRH1 and pregnancy attributed to the new DF formed, which produces fresh oocytes and establishes pregnancy in river buffaloes [17]. Moreover, the presence of DF on Day 7 was found helpful in the present work in identifying timely ovulatory follicles among treated buffaloes, which could have resulted in more precise timing of AI and favored pregnancy in water buffaloes.

Meanwhile, POF Category III yielded the highest pregnancy rate on the Day of AI at Day 10 among POF categories and is within the range of POF size (12.0-16.0 mm) earlier reported in Murrah buffaloes [12]. The authors considered these follicles as mature enough to be transformed into CL and sustain pregnancy by maintaining optimal CL diameter and peripheral progesterone concentration. The POF size on the Day of AI is strongly associated with pregnancy in the present study, which is consistent with previous findings in buffaloes [11,18]. Pfeifer *et al*. [10] supported the above claims with the findings that larger size POF is comprised of a higher number of granulosa cells, which have been related to the greater steroidogenic capacity of the resultant CL. Further, pregnancy was found greater in suckled Bos indicus cows that had a larger follicle on the day of FTAI, and cows with follicles >11.1 mm had an increased display of estrus and pregnancy [9]. In addition, mRNA and proteins were reported to be produced and stored in the oocyte, and developmental competence continues to increase with increased follicular diameter in cattle [19]. In contrast, Perry *et al*. [15] and Perry *et al*. [16] demonstrated in beef heifers that follicles induced to ovulate <11.0 mm size had smaller CL and secreted less progesterone than heifers ovulating in large follicles and consequently resulted in lower pregnancy and increased embryonic loss. Most recently, postponing the timed AI by 24 h in cows with smaller follicles with lower pregnancy improves pregnancy [20]. Therefore, methods that increase the diameter of the large follicles on the day of FTAI may be important to improve fertility in buffalo species.

In terms of the signs of estrus, most of the buffaloes demonstrated intense uterine tonicity of Tone 3 with the highest pregnancy, indicating the efficacy of FTAI protocol in water buffaloes. The strong association between uterine tonicity and pregnancy in the present study could be due to the high steroidogenic activity of the large POF during estrus. The uterine tone has been reported to be essential in cows since the contractions of the myometrium can speed up the transit and storage of sperm in the oviduct, increasing the likelihood of fertilization following insemination [21,22]. On the other hand, mucus discharge was not positively correlated with pregnancy, and this could be due to the inherently poor and less distinct display of mucus discharge in buffaloes, resulting in varied pregnancy outcomes. An earlier study supports the current findings that the ovulatory follicle size is a strong indicator of follicle maturity and a better indicator of fertility than serum concentration of estradiol at the time of AI or expression of estrus in cattle heifer [15,16]. Finally, of the different variables evaluated for their potential to predict pregnancy in the present study, the size of POF at the time of AI and uterine tonicity are found to be significant factors in determining the success of FTAI in water buffaloes. The present work established uterine tonicity, with or without the aid of ultrasound and regardless of the presence or absence of mucus discharge, as a helpful reference for technicians to conduct AI during the CIDR-Synch-hCG protocol in water buffaloes.

The present study highlights a follicle size-based CIDR-Synch-hCG FTAI protocol in water buffaloes, which has huge applications in controlled breeding programs for other livestock species. The above protocol likewise offers new strategies to improve the efficiencies of other assisted reproductive biotechnologies to maximize productivity and profitability in livestock.

CONCLUSION

Based on the study, it is concluded that the size of the Pre-Ovulatory Follicle and uterine tone on the Day of AI play important roles in the outcome of pregnancy following CIDR-Synch-hCG FTAI in water buffaloes. Overall, 44.44% of all buffaloes subjected to the above protocol were pregnant. The POF size ≥12.0 mm and uterine tone of 3 form a strong basis to perform AI and serve as important fertility indicators in water buffaloes. With its strong association with POF size on the Day of AI, the uterine tone can be a useful guide for the technician on the proper timing of AI in the field. Further studies with a greater number of animals are warranted to determine the optimal diameter of POF on the Day of AI to maximize the efficiency of the current protocol. Likewise, POF sizes must be investigated for their association with other factors such as estradiol concentration, ovulation response, and luteal profile to determine their influence on the fertility of buffaloes subjected to CIDR-Synch-hCG FTAI protocol.

AVAILABILITY OF DATA AND MATERIAL

Data and material of this research are available from the main author on request.

CONFLICT OF INTEREST

We hereby declare that there is no conflict of interest with respect to the publication of this manuscript.

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